Impact of Direct LDL-C and HDL-C Method Standardization by US-Japan Joint Meeting on the Japanese Guidelines for Cardiovascular Diseases.

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Introduction

A new assay system for homogenous (Direct) LDL-C measurement, developed by Dr. Sugiuchi and his colleagues was published in Clinical Chemistry in 1998[1]. Several Japanese companies subsequently developed new assay reagents by different principles which were distributed worldwide. Differences in results were observed in analysis of samples from dyslipidemic patients which may reflect fundamental methodological differences among the assays. Specifically, each assay reagent measures different lipid fractions of the lipoproteins, e.g. β-VLDL, apoE-rich HDL, β-HDL, Lp(a) and small dense LDL.

We proposed that Japanese companies and other reagent manufacturers state which kinds of lipoproteins are measured by their reagents, to enable clinical laboratories, scientists and clinicians to standardize and compare different tests. Furthermore, collaboration was sought with laboratory scientists in the United States as well as in Europe on the standardization of tests for HDL-C and LDL-C with those of Japan.

US Japan Joint Meeting for Standardization of Lipoproteins

The first US-JAPAN Joint Meeting for Standardization of Lipoproteins was organized by Dr. Russ Warnick at the time of the AACC Annual Meeting held in Los Angeles 2004. The event was sponsored by The Pacific Biometrics Research Foundation (PBRF,) and The Health Care Technology Foundation in Japan (HECTEF,). The following members were present: Warnick R., Miller G., Myers G., Nakamura M, Nakajima K., Kimberly M, McNamara J., Remaely A., McConnell G., Mallory T, Haefner D., Okazaki M., Leary E.T. plus additional scientists from the US, Japan, and Europe.

Over the course of the following six years, the US-Japan Joint Meeting for Lipoprotein Standardization congregated annually at the AACC Annual Meeting: the second was held in Orlando in 2005, a special US-Japan Joint Meeting was held in 2005 at Fukuoka City in Japan, with guest speaker Dr Russ Warnick, the third meeting was in Chicago (2006), the fourth in San Diego (2007), fifth in Washington DC (2008), and the sixth meeting was in Chicago (2009). The lectures have always been of the highest quality and invaluable to progress in the field. After 7 years, we returned to Los Angeles in 2010.

International Lipoprotein Standardization Forum

Over time, during these meetings, we discussed incorporating the US-Japan Joint Meeting for Lipoprotein Standardization into a more open and public forum as part of the AACC. In 2008, Dr Russ Warnick and Myers G. proposed that The US-Japan Joint Meeting be incorporated into a new organization, the International Lipoprotein Standardization Forum in LVDD. The Japanese group, Nakajima K, Nakamura M. and Sakurabayashi I. were honored to join as inaugural members of the LVDD Executive Meeting.

Data from homogeneous assays for LDL-cholesterol and HDL-cholesterol reagents of 8 manufacturers were measured by the CDC and Berkeley HeartLab using fresh panel samples from Virginia Commonwealth University Medical Center and NIH. We gratefully acknowledge the 8 manufacturers who donated research funding, test reagents and calibrators: Roche (a Kyowa distributer), Sekisui, Denka, Kyowa, Wako, Sysmex, Serotec, and UML.

Publishing the Standardized LDL-C & HDL-C Data

A summary of the homogeneous assay data was reported last year in Clinical Chemistry (June 2010)[2]. The objective of the project was to determine the assay performance characteristics of all current reagent formulations for HDL-C and LDL-C assays, with a focus on dyslipidemic patients using a panel of freshly collected patient samples.

The findings were summarized thus: Homogeneous methods have relatively good performance for non-diseased subjects. However, performance for patients with dyslipidemias was often inadequate, as determined by NCEP performance goals. Specificity for HDL-C and LDL-C lipoprotein fractions was a challenge for all methods for disease subjects.

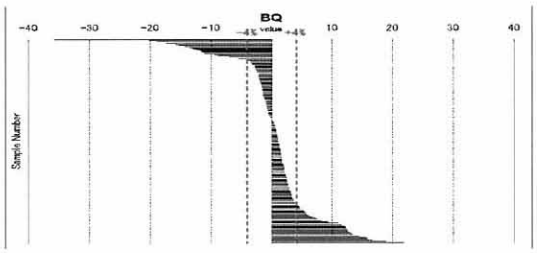
Japanese Data for the Standardization of Lipoproteins program

The data for standardization of LDL-C by the CDC Cholesterol Reference Method Laboratory Network (CRMLN) Program was presented by Dr M. Nakamura at the Annual Meeting of the Japan Atherosclerosis Society, 2009[3]. This study used the LDL Cholesterol Certification Protocol. LDL-C reference values were measured by the beta-quantification method. Analytical guidelines stipulated that the manufacturers perform the LDL-C analysis to within ± 4% bias from the reference values. In order to complete over 5 runs of 40 split sample comparisons,

commonly used among manufacturers, samples were taken from volunteers at the manufacturer's sites, 80% of whom were within the normal range.

The resulting % bias of 7 Japanese reagents is shown in Fig 1: The distribution of % bias of LDL-C in clinical laboratories compared to the CDC/CRMLN method over a three year period. Total cholesterol by 20 analytical systems for normal subjects was within ± 3% of CDC reference criteria. HDL-C by 20 analytical systems for normal subjects was within ± 5% of CDC criteria. TG values were within 5% of CDC criteria. However, LDL-C was > 4% of CDC criteria. In total, 594 samples were measured by 99 clinical laboratories during three years. 70.4% of the samples were within ± 4%. But data varied widely, from -35.8% to +24.5% during the period 2006 to 2008.

Fig.1 Distribution of % Bias of LDL-C in Clinical Labs.



594 subjects measured in 99 Japanese clinical laboratories for 2006 to 2008

f ±4%: 418 subjects, 70.4%
Minimum %bias: -35.8% (-52.5 mg/dL)
Maximum %bias: +24.5% (+32.3 mg/dL)

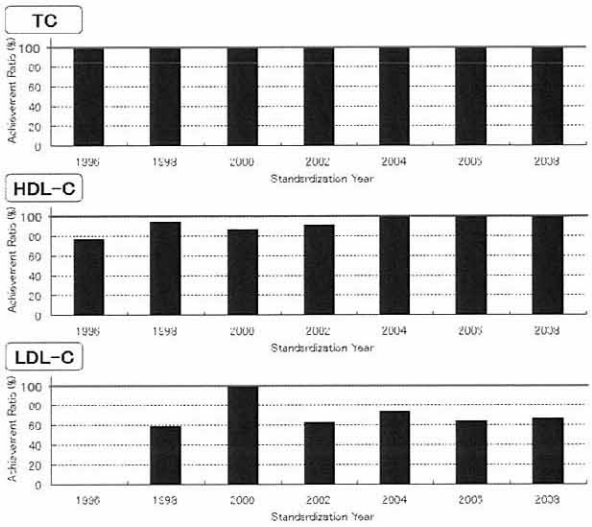
Also, over a 12 year period, at 2 yearly intervals, Nakamura M. examined the total cholesterol, HDL-C, and LDL-C achievement ratio by CDC criteria. From 1996, total cholesterol achieved 100% by all attended reagents. For HDL-C, 100% achievement of CDC criteria was attained by 2004 (Fig 2). However, although LDL-C achieved 100% of CDC criteria in 2000, this was not maintained through 2008. To our knowledge only 3 reagents were in use in the Japanese market in 2000, hence the subsequent failure to maintain 100% achievement may be due to the entry of other manufacturers' reagents into the LDL-C measurement arena.

Nakamura M. concluded that the Japan Atherosclerosis Society guidelines for the Prevention of Atherosclerosis should recommend routine measurement of total cholesterol and HDL-C, together with a increased emphasis on special health care checkups focused on metabolic syndrome. For LDL-C, long-term stability and further improvement of accuracy would be required.

Diagnostic criteria of dysplidemia in the Japan Atherosclerosis Society

Dyslipidemia is diagnosed as ≥140mg/dl of LDL-C, <40mg/dl of HDL-C or ≥150 mg/dl of

Fig. 2 Achievement ratio of standardization by CDC criteria.



triglyceride (see Table 1). The Japanese guidelines also recommend the following: when TG is >400mg/dl or the sample is from the non-fasting state, the LDL-C should be determined by direct measurement.

Table 1. Diagnostic Criteria for Dyslipidemia (Serum Samples after Overnight Fasting) in 2007

LDL-Cholesterol	≥	140mg/dl
HDL−cholesterol	<	40mg/dl
Triglyceride	≥	150mg/dl

Diagnosis of dyslipidemia is made when either type of lipid abnormality is present.
These diagnostic criteria are not intended for the beginning of drug therapy.
It is important to consider the indications of drug therapy only after evaluation of other risk factors.
LDL-C is evaluated basically by calculation with the Friedewald equation .
When the TG is ≥ 400mg/dl or non-fasting state, the LDL-C should be determined by direct measurement

Lipid management goal in Japan

Japanese guidelines for "Lipid Management Goals based on Risk Assessment" were published by the Japan Atherosclerosis Society in 2007 (Table 2). Patients are classified into 4 categories according to LDL-C levels. In the primary prevention category were three classes, in Class I there is no major risk factor

other than LDL-C, and lipid management goals are to achieve LDL-C concentration <160mg/dl, HDL-C concentration ≥40mg/dl and TG levels <150mg/dl. Class II (intermediate risk) has an LDL-C management goal of <140mg/dl and Class III (high risk) <120mg/dl. Class IV is the patient with a history of coronary diseases and an LDL-C management goal of <100mg/dl. These 4 categories are classified only by LDL-C, total cholesterol is not considered.

Table 2. Japanese Guideline for the Lipid Management Based on Risk Assessment in 2007

Lipid management goals based on risk assessment					
Principle of therapeutic strategy	category		Lipid management goals (mg/dL)		
		Major risk factors other than LDL-C*	LDL-C	HDL-C	TG
Primary prevention Lifestyle should be changed before consideration of drug therapy	I (Low-risk group)	0	<160	≥ 40	< 150
	II (Intermediate-risk group)	1–2	< 140		
	III (High-risk group)	3 or more	< 120		
Secondary prevention Both drug therapy and lifestyle modification are considered	History of coronary artery diseases		< 100		

Management of serum lipids as well as intervention of other risk factors (smoking, hypertension or diabetes) is necessary.
*Major risk factors other than LDL-C
Aging (male ≥ 45 years, female ≥ 55 years), hypertension, diabetes (including impaired glucose tolerance), smoking, family history of coronary artery disease, low HDL cholesterol (< 40 mg/dL)
· Category III, if complicated by diabetes mellitus, cerebral infarction or arteriosclerosis obliterans.

Japanese Special Health Care Examination Program

The Japanese Special Health Care Examination Program was launched nationwide in 2008. The target disease of particular focus was metabolic syndrome. The Ministry of Health, Labor and Welfare selected diagnostic laboratory testing for LDL-C, HDL-C, TG (without total cholesterol), as well as height, body weight, BMI, abdominal girth, blood pressure etc.

Total cholesterol was excluded for two reasons: the increase in number of individuals in Japan having high HDL-C concentration and health insurance. Firstly, after World War II, Japanese eating habits changed from the traditional Japanese diet to westernized food. However, the Japanese and Asians in general have a genetic isoform of the cholesterol ester transfer protein gene (*CETP*) that produces a protein with reduced ability to transfer cholesterol to other lipoproteins. In such individuals, on a cholesterol rich diet, cholesterol accumulates in the HDL particle and increases serum HDL-C levels. Unfortunately, measuring total cholesterol in such individuals may lead to an incorrect diagnosis of hyperlipidemia and treatment with inappropriate medications.

Secondly, only three items can be selected from total cholesterol, LDL-C, HDL-C and TG for testing dyslipidemic patients according to the National Health Insurance Act. If more than three tests are selected, payment is denied by the National Payment Foundation. For this reason LDL-C and HDL-C measurements are most suitable for the screening of dyslipidemic patients.

Statement of the Japan Atherosclerosis Society

In April of this year, the Japan Atherosclerosis Society released a statement to the press about the guidelines and LDL-C measurement. Teramoto T, the chairman of "the Japanese Guideline for Lipid Management Based on Risk Assessment", warned that the LDL-C direct method has not yet achieved satisfactory performance. The accuracy of the direct method is still suboptimal and the Friedewald equation is fundamental for the fasting condition. Clearly, this statement was influenced by our report published in Clinical Chemistry, 2010[2].

Kita T, the Chairman of the board of trustees of the Japan Atherosclerosis Society, announced the following in the same press release:

1) The Society strongly believes that the reagents need better standardization, higher precision and clearer information is needed about performance of the LDL-C direct methodology.
2) Although total cholesterol was not included in past Japanese Health Care examinations, this measurement should be added to the Special Health Care Examination Program.
3) In the case of patients with high TG concentration, non-HDL-C calculated by total cholesterol and HDL-C should be considered.

Finally, Dr. Kita emphasized that LDL-C direct methods will be used in health care examinations and diagnosis of dyslipidemic patients if satisfactory progress is made towards improving the accuracy of these reagents.

Requirement of the Ministry of Health, Labor and Welfare

Recently, the Ministry of Health, Labor and Welfare requested a new study: There are 12 commercially available LDL-C reagents already approved by the Ministry including OEM kits which are not standardized. The Japan Association of Clinical Reagents Industries were asked to perform a standardization using the secondary reference method of HECTEF, approved by the Japanese Society of Clinical Chemistry. The beta-quantification method and secondary reference method were to be performed by Nakamura M. (CDC Lab in Osaka) and Kayamori Y. (Kyushu-University).

This secondary reference method for LDL-C uses ultracentrifugation to remove VLDL and a dextran sulfate-magnesium precipitation method to separate HDL, followed by an enzymatic UV method for cholesterol measurement[4]. This reference method was approved by the Japanese Society of Clinical Chemistry in 2009[5].

The chairman of this project was nominated to be Miida T. (Juntendo University), who spoke about pre-beta HDL-1 at the LVDD dinner party 2 years ago. Murakami T. (Gunma University), along with other clinical laboratory scientists and clinicians, will take charge of gathering more than 100 dyslipidemic samples. At the end of this year, the results of the study on the 12 Homogenous LDL-C reagents will be published and comments invited by the Ministry.

Conclusion

We believe the US-Japan joint scientific work on direct LDL-C and HDL-C method standardization could much improve the national organization of lipid testing in Japan. We hope homogenous HDL-C and LDL-C reagents improve the accuracy and long term stability of these tests in the future and allow for more widespread use of homogeneous HDL-C & LDL-C reagents.

References

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